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(54) Title: ASSAY FOR THE DETECTION OF SPECIFIC LIGANDS

(57) Abstract

The invention disclosed herein relates to the field of one-step assays and particle agglutination tests. In one embodiment of the present invention, there is disclosed an analytical device for the detection or determination of an analyte antibody in a bodily fluid comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, the layer including a sample application zone, a conjugate zone containing antigen bound to mobile particles, and a detection zone containing immobilized antigen, wherein the antigen is the same in both the conjugate and detection zones and is an antigen that binds with the analyte antibody, the liquid sample is capable of moving from the sample application zone through the conjugate zone and on to the detection zone, and if the analyte antibody is present in the sample it is detected in the detection zone. The present invention also discloses methods of detecting various analytes, particularly analyte antibodies, including specific immunoglobulins. The invention further provides improved methods of preparing and using one-step assays, as well as improved methods of preparing coated particles for use in diagnostic assays.

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ASSAY FOR THE DETECTION OF SPECIFIC LIGANDS

RELATION TO RELATED APPLICATION

This application is a continuation-in-part of the U.S. Patent Application, serial no. unknown, filed June 13, 1991, naming Tzeng, et al., as inventors. Priority of subject matter in this application common with subject matter in that application is hereby claimed.

BACKGROUND OF THE INVENTION

Recent technological advances have made it possible to tailor assays for a wide variety of analytes, especially those molecules exhibiting antigenic characteristics, such as polypeptides, nucleotides, whole cells, and cellular fragments, to name but a few. In general, most assays currently in use tend to use antibodies to "capture" antigenic materials in a liquid-phase or a solid-phase format.

Nevertheless, assays with an improved sensitivity are needed, as many conditions and diseases do not lend themselves to early diagnosis via antigenic detection. In many instances, it would be preferable to be able to detect an increase in a specific population of cells or molecules in an organism, which population is produced in response to an "invasion" of the organism by a specific antigenic material.

For example, in the case of certain viral diseases, by the time a sufficiently detectable titer of viral particles is present in an organism's blood, the time for effective therapy may well have passed. In addition, diseases that trigger highly specific, but virtually undetectable, responses by an organism's immune system, do not lend themselves to accurate or easy detection via currently-available assays. Moreover, agents which provoke a response by a specific population or subpopulation of immunoglobulins are often not detectable until the resulting disease is full-blown, thus limiting diagnostic, as well as therapeutic, options. For example, an assay claiming to facilitate the differential detection of

various immunoglobulin species from each other is described in U.S. Patent No. 4,292,403 to Duermeyer. Specifically, this assay purports to detect an antigen-specific immunoglobulin of a particular immunoglobulin class, which classes include IgM, IgG, IgA, IgD, or IgE, by using anti-antibodies against the specific immunoglobulin class. Like many other assays disclosed in the art which use anti-antibodies (e.g., U.S. Patent No. 4,818,688 to Adamich, et al., No. 4,828,981 to Maggio, et al., and No. 4,962,023 to Todd, et al.), the assay described by Duermeyer is complex and involves a multiplicity of specific reagents. Furthermore, none of these assays can easily distinguish an antigen-specific subpopulation of an immunoglobulin class from other, non-antigen-specific members of its class. That is, prior to the advent of the present invention, it has not been a simple matter to detect subpopulations of specific IgG or IgM molecules, for example, which are precisely sensitized to a single antigenic substance.

An illustrative example of the need for assays with increased sensitivity is provided by consideration of available assays for the detection of antibodies, and in particular, for the detection of immunoglobulins directed against specific antigens. Lyme Disease provides such an illustrative example.

Lyme Borreliosis was first identified in Lyme, Connecticut in 1975. It has now been reported across several continents, including North America, Europe, Russia, Asia, Africa and Australia. The disease is caused by the tick-transmitted spirochete *Borrelia burgdorferi*. This infection can produce a wide spectrum of clinical symptoms which can be confused with other entities. Therefore, precise diagnosis is critical.

Lyme disease is the most commonly reported tick-borne illness in the United States. The disease is most prevalent

in the northeast, upper midwest, and west coast states. The seasonal onset of the disease is synchronous with the nymphal stage of the ticks. Thus, the disease is more common in summer and early fall.

Antibody titers specific to *Borrelia burgdorferi* are typically negative during early illness. Patients with only erythema migrans also rarely have elevated antibody titers. The specific IgM levels begin to rise two weeks after the onset of the disease and peak at three to six weeks. The specific IgG levels tend to lag behind the specific IgM titers by approximately two weeks, but are often positive during the latter part of the clinical stage of erythema migrans and usually remain positive during the second and third clinical stages with manifestations of carditis, neurological disease or arthritis.

Generally, the "natural history" or progression of Lyme disease may be divided into three clinical stages. The first stage is characterized by the development of an expanding annular red rash-like skin lesion, erythema migrans, which occurs at the site of the tick bite and typically lasts two to four weeks. The erythema migrans may be followed by cardiac, joint and neurological abnormalities in the second stage, which occurs one to four months after the disease onset. The last stage is characterized by arthritis involving a few large joints which may begin as early as three months after onset. This stage can last several years or may become chronic. Early diagnosis permits prompt treatment with appropriate antibiotics that can halt the progression of the disease.

Since the spirochete is often difficult to culture from affected skin or body fluids of patients, serological detection of antibodies is considered the best available diagnostic means for Lyme disease. The specific IgM against *Borrelia burgdorferi* is often not detectable during the first two weeks, but it usually peaks three to six weeks after the initial infection, and then persists or declines. The

response of the specific IgG to the spirochete is frequently not detectable for four to six weeks, but peaks in the arthritic stage and often remains elevated for years.

Therefore, in response to an express need for an assay procedure with diverse applicability, which is capable of detecting specific ligands, including various polypeptides, polynucleotides, and immunoglobulins, with great sensitivity, Applicants disclose the present invention. In addition, the presently-described assays avoid the agglomeration problems of other assays, which promotes the goals of improved accuracy and greater resolution. The present invention, which is elegant in its simplicity, is hereby disclosed by Applicants, including its equivalents thereof.

SUMMARY OF THE INVENTION

In one embodiment of the present invention, Applicants disclose an analytical device for the detection or determination of an analyte antibody in a bodily fluid comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, the layer including a sample application zone, a conjugate zone containing antigen bound to mobile particles, and a detection zone containing immobilized antigen, wherein the antigen is the same in both the conjugate and detection zones and is an antigen that binds with the analyte antibody, the liquid sample is capable of moving from the sample application zone through the conjugate zone and on to the detection zone, and if the analyte antibody is present in the sample it is detected in the detection zone.

In another embodiment, the mobile particles are colored plastic particles or a metal sol. Another variation discloses a device wherein the antigen will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism. In another variation, the mobile particles are colored polystyrene microparticles. In yet another embodiment, the layer is made from nitrocellulose.

The present invention also discloses a method employing the disclosed devices, which comprises adding sample suspected of containing the analyte antibody to the sample application zone and waiting for sufficient time for the sample to traverse the layer through the detection zone, and reading the results in the detection zone. In one variation, the mobile particles are colored plastic particles or a metal sol. In another variation, the antigen used will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism. Yet another aspect discloses that the mobile particles are colored polystyrene microparticles. In another embodiment, the layer is made from nitrocellulose.

The present invention further discloses a process for the determination of the presence or concentration of an analyte antibody in a sample fluid which comprises contacting a sample of the fluid with a first antigen for the analyte antibody, wherein the first antigen is labelled, in order to form a soluble complex between the first antigen and the analyte antibody; contacting the soluble complex with a second antigen, wherein the second antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex of the first antigen, the analyte antibody, and the second antigen; separating the solid phase from the fluid sample and the unreacted, first antigen; measuring either the first, labelled antigen associated with the solid phase of the unreacted amount of the first, labelled antigen; relating the amount of first, labelled antigen measured for a control sample prepared in accordance with the first four steps, the control sample being free of the analyte antibody, to determine the presence of the analyte antibody in the fluid sample, or relating the amount of first, labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with the first four steps in order to determine the concentration of the analyte antibody in the fluid sample; wherein both the first and

second antigen are the same before they are labelled or attached to the solid phase; respectively. Those skilled in the art will realize that, in the case where the label is a visible particle, such as a gold sol or colored or colorable microparticle, the complex formed by the labelled antigen and the analyte antibody may be insoluble.

In yet another embodiment, the label is an enzyme or a radioisotope. In another alternative embodiment, the antigen reacts with antibodies that in turn react with epitopes of *Borrelia burgdorferi*. Other variations disclose that the label is alkaline phosphatase and the solid phase is a bead, the inner walls of a test tube or the wells of a microtitre plate; alternatively, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

The present invention further discloses an assay kit comprising a first antigen bound to a solid phase insoluble in the fluid to be tested and a reagent containing a second antigen bound to a label, wherein the solid phase and the reagent are present in sufficient amount to perform at least one assay for analyte antibody in the fluid, and wherein the first and second antigen are the same before they are bound to the solid phase or labelled, respectively. In another variation, the label is an enzyme or a radioisotope. In yet another embodiment, the antigen is an antigen that binds to antibodies which in turn bind to epitopes of *Borrelia burgdorferi*. Another variation discloses that the solid phase is a bead, the inner walls of a test tube or a non-chromatographic apparatus, and the label is alkaline phosphatase. In another embodiment, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

In another embodiment, an assay kit is disclosed, which comprises a first antigen bound to a solid phase insoluble in the fluid to be tested and a reagent containing a second antigen bound to a label, wherein the solid phase and the reagent are present in sufficient amounts to perform at least

one assay for analyte antibody in the fluid, and wherein the first and second antigens are the same before they are bound to the solid phase or are labelled, respectively.

In another embodiment, a process is disclosed, whereby the process is for the determination of the presence or concentration of an analyte antibody in a fluid comprising the following steps: a) simultaneously contacting a sample of the fluid with a first and second antigen, wherein the first antigen is bound to a solid phase insoluble in the fluid and the second antigen is labelled and provided in a measured in order to form a insoluble complex between the first and second antigens and the analyte antibody; b) separating the solid carrier from the fluid sample containing unreacted second, labelled antigen; c) measuring the amount of the second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen; d) relating the amount of second labelled antigen with the amount of labelled antigen measured for a control sample prepared in accordance with steps (a) through (c), the control sample known to be free of analyte antibody, to determine the presence of analyte antibody in the fluid sample, or relating the amount of labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amount of analyte antibody prepared in accordance with steps a) through c) to determine the concentration of the analyte antibody in the fluid sample; wherein the first antigen and second antigen are the same before they are bound to the solid phase or labelled, respectively.

In alternative embodiments, the label is an enzyme or radioisotope; further, the first and second antigen may bind to antibodies that in turn bind to epitopes of *Borrelia burgdorferi*. In another variation, the label is alkaline phosphatase and the solid phase is a plastic bead, the inner walls of a test tube, the wells of a microtitre plate. In yet another embodiment, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

Another embodiment discloses a process for the determination of the presence or amount of an analyte antibody in a fluid sample comprising the steps of: a) contacting a sample of the fluid with a first antigen, wherein the first antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex between the first antigen and the analyte antibody; b) separating the fluid sample containing the unreacted analyte antibody from the insoluble complex of the analyte antibody and first antigen; c) reacting a measured amount of a second, labelled antigen with the insoluble complex of the first antigen and the analyte antibody to form an insoluble complex composed of the first and second antigens and the analyte antibody; d) separating the solid phase from the unreacted second, labelled antigen; e) measuring either the amount of second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen;

f) relating the amount of second, labelled antigen measured with the amount of labelled antigen measured for a control sample prepared in accordance with steps a) through e) being known to be free of the analyte antibody to determine the presence of analyte antibody, or relating the amount of labelled antigen measured in the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with steps a) through e) to determine the concentration of analyte antibody in the fluid sample; wherein the first and second antigens, before they are bound to the solid phase or labelled, respectively, are the same.

In various alternative embodiments, the label is an enzyme, a radioisotope, or alkaline phosphatase. In another variation, the first and second antigens are both antigens that complex with epitopes of the *Borrelia burgdorferi* microorganism. Yet another embodiment discloses a process

wherein the solid phase is a bead, the inner walls of a test tube, or the wells of a microtitre plate; in another variation, the solid phase is a non-chromatographic device.

DETAILED DESCRIPTION

The present invention is directed to devices and methods for simply and rapidly performing assays that are capable of differentiating specific molecules, such as antibodies. While any convenient solid material may be used as a solid support, it is preferable to use any one (or more) of several bibulous or absorbent solid materials, which may be employed to allow for capillary transport of a liquid away from the interface between the air and liquid. Various bibulous materials include paper, cellulose particles, silica gel, cellulosic beads, glass fiber, filter paper, and the like. The surface of the solid support should be relatively smooth, so as to allow for the formation of a concentrated particle site, for example, in the form of a sharp band or point. The size and shape of the bibulous material may be varied widely depending upon the purpose of the assay. For example, the bibulous material may be shaped as a narrow strip of from about one to about five millimeters in width, or it may be in any other convenient geometric or non-geometric shape. In each case, the bibulous material will usually have a support which provides structural strength. The non-bibulous material may be a water impermeable layer or coating.

One embodiment of the present invention has the antigens as capture antigens bound to a porous membrane. A porous membrane may be comprised of a flexible or rigid matrix made from any of a variety of filtration or chromatographic materials including glass fibers, micro-fibers, and natural or synthetic materials. Fluids should be able to flow into and pass easily through the porous membrane. The membrane should also preferably have pore sizes of at least 0.1μ and preferably no more than 20μ. The porous membrane can be used alone or as part of a more elaborate device. Such devices

include the non-chromatographic ICON® and like devices described in Valkirs, et al., U.S. Pat. Nos. 4,632,901 and 4,727,019, issued December 20, 1986 and February 23, 1988, respectively, herein incorporated by reference. ICON® is a trademark of Hybritech Incorporated (San Diego, CA) for the devices described in the Valkirs, et al. patents listed above.

More specifically, Valkirs, et al. describe an apparatus for the detection of a target antigen in a liquid sample, comprising: (a) a first member which is a porous membrane or filter and to which is bound an antibody against the target antigen, which member has upper and lower surfaces, the sample being applied to the upper surface, and wherein the antibody is bound within an area smaller than the area of the member to which the sample is applied; and (b) a second member, which is a body of absorbent material having a surface over which the first member is placed and having capillaries therethrough in a direction generally transverse to the surface over which the first member is placed, which capillaries are in communication with the pores on the lower surface of the first member so as to draw liquid added to the upper surface which has permeated the first member into the capillaries of the member, the capillary communication between said first and second members having been established prior to, and maintained during, the addition of liquids to the apparatus in the immunoassay process.

Another such device is the TestPack® device of Abbott Laboratories (North Chicago, IL), described in European Patent Application No. 217,403, published April 8, 1987. Still other devices containing porous membranes useful in the present invention include the devices of Bauer, et al., U.S. Pat. No. 3,811,840, issued May 21, 1974; Brown, III, et al., U.S. Pat. No. 4,916,056, issued April 10, 1990; Cole, et al., U.S. Pat. No. 4,407,943, issued Oct. 4, 1983; Cole, eg al., U.S. Pat. No. 4,246,339, issued Jan. 20, 1981; Intengan, U.S. Pat. No. 4,440,301, issued April 3, 1984; Jolley, U.S. Pat. No. 4,704,255, issued Nov. 3, 1987; Katz, et al., U.S. Pat. No. 4,496,654, issued Jan. 29, 1985; and Tom, et al., U.S. Pat.

No. 4,366,241, issued Dec. 28, 1982, all of which are incorporated herein by reference.

The present methods also may be accomplished by chromatographic methods such as, for example, those described in Weng, et al., U.S. Pat. No. 4,740,468, issued April 26, 1988, incorporated herein by reference, and published European Application No. 186,100 to Yue, et al., published July 2, 1986.

The porous membrane of the present invention may also be used in chromatographic assays described, for example, in U.S. Pat. No. 4,861,711, issued August 29, 1989 to Friesen, et al.; U.S. Pat. No. 4,855,453, issued August 8, 1989 to Rosenstein, et al.; U.S. Pat. No. 4,857,453, issued August 15, 1989 to Ullman, et al., all of which are incorporated herein by reference, and May, et al., EPO Publication No. 291,194, published Nov. 17, 1988; Ching, et al., EPO Publication No. 299,428, published Jan. 18, 1989, and Devereaux, et al., EPO publication No. 323,605, published July 12, 1989.

The capture antigens may be directly or indirectly bound to the membrane. The direct binding may be covalent or non-covalent and may be accomplished by any method known in the art such as, for example, the use of glutaraldehyde and aminosilanes as well as other methods described in "Immobilized Enzymes", Ichiro Chibata, Halstead Press, NY (1978); Cuatrecasas, J. Bio. Chem. 245: 3059 (1970); and March, et al., Anal. Biochem. 60: 149, et seq. (1974). The non-covalent binding takes advantage of the natural adhesion of antibodies to the non-synthetic and especially the synthetic fibers. Thus, appropriately buffered solutions can be mixed with the membrane then evaporated, leaving a coating of the desired ligand on the membrane.

The non-direct method for applying the ligand to the membrane employs microparticles that may be bound to or entrapped by the membrane, such that the microparticles are within the matrix of the membrane, on the surface of the membrane, or bound to other particles which are in turn bound to the membrane. The microparticles may be any shape,

preferably spherical. The size of the particles should be such that they do not migrate through the membrane to any significant degree. The size of the particles may vary, but in general, they may be slightly larger than the minimum pore size of the membrane and smaller than the maximum pore size, and in addition or in the alternative, may be larger than the maximum pore size. Thus, the particle may be bound within the matrix of the membrane, on the surface of the membrane, or to other particles which are in turn bound to the membrane. The particles may be made of a variety of naturally occurring or synthetic materials. Exemplary of such particles are those made from polyethylene, polyacrylates, polyacrylamide, and preferably polystyrene or naturally occurring materials such as cross-linked polysaccharides like agarose, dextran, cellulose, starch, or the like. The primary requirement is that the particles do not contribute a signal, usually light absorption, that would cause the zone in which the particles were located to have a different signal than the rest of the membrane.

The ligand may be covalently or non-covalently bound to the microparticle. The binding of ligand to the particle may be by methods similar to those discussed above for binding the ligand directly to the membrane or other methods known to those skilled in the art. The preferred method for coating ligands to the microparticles is described in the Examples herein.

The particles may be applied (or "spotted") to the membrane in a zone within the surface area of the membrane. Thus, spotting localizes the antigen-coated microparticles to a discrete area on the membrane to localize the antigen-coated microparticles on or within the membrane. Any of the methods known in the art may be employed. One such method employs various mechanical means such as, for example, the Sandy Springs Spotting Machine (Germantown, MD) to apply a suspension, frequently aqueous ("latex"), to the membrane.

The methods of preparing and using such microparticles for the instant invention are further discussed in Weng, et

al., U.S. Pat. No. 4,740,468, issued April 26, 1988 (see especially columns 13-15) incorporated herein by reference; Brown, III, et al., European Patent App. No. 217,403, published April 8, 1987, ; and A.S. Rubenstein, European Patent App. No. 200,381, published Nov. 5, 1986.

The separation steps for the various assay formats (e.g.,, forward, simultaneous, and reverse) may be performed by any of the methods known in the art. For membranes and filters, additional washing with buffer may often be sufficient, preferably drawing the liquid through the membrane or filter by applying vacuum to the opposite side of the membrane or filter or contacting the opposite side of the filter or membrane with a liquid absorbing member that draws the liquid through, for example, via capillary action. The ICON® device (Hybritech, Incorporated, San Diego, CA), which is preferred for use in one embodiment of the present invention, uses the latter method.

In addition, non-chromatographic devices and assays are appropriate for use according to the present invention. Bead assays, for example, provide an alternative to chromatographic assays. See, e.g., Geigel, et al., U.S. Pat. No. 4,517,288, issued May 14, 1985, which is incorporated herein by reference.

Moderate temperatures are normally employed for carrying out the assay. Constant temperatures during the period of the measurement are generally required only if the assay is performed without comparison with a control sample. The temperatures for the determination will generally range from about 15°-45°C.

The term "labeled antigen" refers to any antigen having specific reactivity with the particular antibody of interest. Such an antigen may be labeled by conventional methods to form all or part of a signal generating system. For example, the antigen may be labeled with radioactive isotopes, enzymes, biotin, avidin, chromogenic or fluorogenic substances,

chemiluminescent labels, colloidal metal particles, colored microparticles, colorable particles, and colorable latex particles.

Thus, the labelled antigen may be covalently bound to radioisotopes such as tritium, carbon 14, phosphorus 32, iodine 125, and iodine 131 by methods well known in the art. For example, I^{125} can be introduced by procedures such as the chloramine-T procedure, enzymatically via the lactoperoxidase procedure, or by the prelabeled Bolton-Hunter technique. These techniques plus others are discussed in h. Van Vunakis and J.J. Langone, eds., Methods in Enzymology 70: Part A (1980). See also U.S. Pat. Nos. 3,646,346, issued Feb. 29, 1972, and 4,062,733, issued Dec. 13, 1979, incorporated herein by reference, for further examples of radioactive labels. Chromogenic labels are those compounds that absorb light in the visible ultraviolet wavelengths. Such compounds are usually dyestuffs and include quinoline dyes, triarylmethane dyes, phthaleins, insect dyes, azo dyes, anthraquimoid dyes, cyanine dyes, and phenazoxonium dyes.

Fluorogenic compounds include those which emit light in the ultraviolet or visible wavelength subsequent to irradiation by light. The fluorogens can be employed by themselves or with quencher molecules. The primary fluorogens are those of the rhodamine, fluorescein and umbelliferone families. The method of conjugation and use for these and other fluorogens can be found in the art. See, for example, Langone and Van Vunakis, Methods in Enzymology 74: apart C (1981), especially at pages 3-105. For a representative listing of other suitable fluorogens, see Tom, et al., U.S. Pat. No. 4,366,241, issued Dec. 28, 1982, especially at columns 28 and 29; and U.S. Pat. No. 3,996,345, both of which are incorporated herein by reference.

Chemiluminescent labels may also be used in the present invention. For example, the labels listed in Maier, et al., U.S. Pat. No. 4,104,029, issued August 1, 1978, herein incorporated by reference, may be used as detection signals in the present invention.

Alternate methods of detection include the use of colored microparticles, colorable particles, including colorable latex particles and colloidal metal particles. Colored microparticles and their use in assays known in the art include, for example, those described in U.S. Pat. No. 4,703,017, issued Oct. 27, 1987 to Campbell, et al., incorporated herein by reference. The use of colloidal metal particles in assays is also well known in the art. See, for example, U.S. Pat. No. 4,313,734, issued Feb. 2, 1982 to Leuvering; U.S. Pat. No. 4,775,636, issued Oct. 4, 1988 to Moermans, et al.; both of which are incorporated herein by reference, and by Yost, et al., EPO Pub. No. 298,368, published Jan. 11, 1989. Colorable particles and colorable latex particles are also known in the art to be useful as markers and are described, for example, in U.S. Pat. No. 4,373,932, issued Feb. 15, 1983 to Gribnau, et al., and U.S. Pat. No. 4,837,168, issued June 6, 1989 to deJaeger, et al., respectively, both of which are incorporated herein by reference.

These non-enzymatic signal systems are adequate for the present invention. However, those skilled in the art will recognize that an enzyme-catalyzed signal system is in general more sensitive than a non-enzymatic system and is, therefore, preferred. Catalytic labels are well known in the art and include single and dual ("channeled") enzymes such as alkaline phosphatase, horseradish peroxidase, luciferase, β-galactosidase, glucose oxidase, lysozyme, malate dehydrogenase, glucose-6-phosphate dehydrogenase, and the like. Dual catalytic systems include, for example, alkaline phosphatase and glucose oxidase using glucose-6-phosphate as the initial substrate. A second example of a dual catalytic system is illustrated by the oxidation of glucose to hydrogen peroxide by glucose oxidase, which hydrogen peroxide would react with a leuco dye to produce a signal generator. A more detailed discussion of catalytic systems can be found, for example, in U.S. Pat. No. 4,366,241 to Tom, et al.,

particularly columns 27-40; U.S. Pat. No. 4,843,000, issued June 27, 1989 to Litman, et al.; and U.S. Pat. No. 4,849,338, issued July 18, 1989 to Litman, et al., all of which are incorporated herein by reference. Also, see Weng, et al., U.S. Pat. No. 4,740,468, which is also incorporated herein by reference, especially at columns 2 and 6-8.

The procedures for coupling enzymes to the antigens are well known in the art and are described, for example, in Kennedy, et al., Clin. Chim. ACTA 70: 1 (1976). Reagents that may be used for this procedure include, for example, glutaraldehyde, p-toluene diisocyanate, various carbodiimide reagents, p-benzoquinone m-periodate, N,N'-o-phenylenedimaleimide and the like.

The substrates for the catalytic systems include simple chromogens and fluorogens such as para-nitrophenyl phosphate (PNPP), β-D-glucose (plus possibly a suitable redox dye), homovanillic acid, o-dianisidine, bromocresol purple powder, 4-alkyl-umbelliferone, luminol, para-dimethylaminolophine, paramethyloxylophine, and the like, with indoxyl phosphate being the preferred substrate.

Depending on the nature of the label and catalytic signal producing system, a signal can be detected by irradiating with light and observing the level of fluorescence; by providing for a catalyst system to produce a dye, fluorescence, or chemiluminescence, where the dye can be observed visually or in a spectrophotometer and the fluorescence could be observed visually or in a fluorometer; or in the case of chemiluminescence or a radioactive label, by employing a radiation counter. Where the appropriate equipment is not available, it will normally be desirable to have a chromophore produced which results in a visible color. Where sophisticated equipment is involved, any of the techniques is applicable. For example, when the preferred combination of alkaline phosphatase is used as the enzyme and indoxyl phosphate as the substrate, a color change may be detected visually for a qualitative positive reaction. For a

quantitative analysis, the ICON® reader and accompanying software (Hybritech Incorporated, San Diego, CA) may be used according to the manufacturer's instructions and are preferred for use in the present invention.

While latex particles tend to be preferred for use in conjunction with the presently-disclosed invention, it should be appreciated that other particles may be used successfully. For example, the particles which are involved in the assay may be present in the sample, may be added as reagents or formed *in situ*. The nature of the particle may vary widely, being naturally occurring or synthetic, being a single material, a few materials, or a combination of a wide variety of materials. Naturally-occurring particles include nuclei, mycoplasma, plasmids, plastids, mammalian cells, unicellular microorganisms (e.g., bacteria). Synthetic particles may be prepared from synthetic or naturally occurring materials, such as metal colloids or latex particles made from polystyrene polyacrylates or naturally-occurring materials, such as polysaccharides, e.g., agarose, or the like. Non-naturally-occurring particles may be varied depending upon the particular assay, the protocol for the assay, or other considerations. (See, e.g., Gould, et al., U.S. Pat. No. 4,837,168, which describes the use of a variety of particles.)

Uniform latex particles ("ULPs") are, in general, extremely uniform spheres of small diameter. Typical diameters range from less than about 0.1μm to about 100 μm. Particles smaller than 5 μm are usually prepared by emulsion polymerization. The result of this process is a series of particles with extremely uniform size distributions.

The principal use for ULPs is in the medical diagnostic area, wherein the particles are utilized for latex agglutination tests. Other varieties of particles, such as amide-modified latex ("AML") and carboxylate-modified latex ("CML") have amide and carboxylic acid groups, respectively,

on their surfaces. These functional groups permit covalent binding of ligands -- for example, antigens or antibodies -- to the surface of the ULPs for improved agglutination tests.

For example, if one is attempting to measure a particular antibody ("Ab"), an appropriate antigen ("Ag") is coated onto the latex particles. Since the Ab is divalent, it may bind to identical sites on two adjacent particles and link them together. Thus, if Ab present in an individual's sample is mixed with the Ag-coated particles, it will cause agglutination or coagulation of the particles; these aggregates are generally visible to the naked eye. This phenomenon is, essentially, the basis for latex agglutination tests ("LAT").

One major difficulty with mobile particles is the fact that the coated particles tend to spontaneously agglutinate. Latex suspensions, in particular, are colloidal suspensions of hydrophobic particles. The stability of the suspension is dependent upon the surface active charges; addition of small amounts of protein (approximately 10 μg per mg of latex) can cause agglomeration, whereas continued addition of larger amounts of protein tends to increase particle stability. This type of agglutination is also a problem in the chromatographic assays using colored or visible particles.

Various methods of addressing this problem and the related problem of nonspecific agglutination have been suggested, including the use of linkers and spacers. However, few of these suggestions prove entirely satisfactory, as they tend to interfere with the assay, many doing so in a manner that inhibits agglutination. This is, of course, a completely unacceptable result for most assays.

The means for detecting a detectable signal at or away from the concentration site may or may not be an intrinsic property of the particles. The particles may be labeled with a wide variety of materials which allow for detection, such as radionuclides, dyes, fluorescers, enzymes, or other convenient label providing for a detectable signal, either visually

observable or detectable by instrumentation. The various labels would normally be covalently bonded to the particle, using linking arms as appropriate. The labels may be bound to the surface or, when feasible, extend throughout the particle.

The size of the particles may vary widely, generally ranging from about 0.05 to 100 micrometers (μm), more usually from about 0.1 to 75μm. The particles may be charged, either positively or negatively, may be amphoteric or lack any charge, being neutral. The presence or absence of charge may affect other parameters involved in the assay.

A large number of patents have been issued which describe a wide variety of labels which have found use in diagnostic assays. Various protocols can be developed where these labels may be used with advantage. Illustrative of such patents are U.S. Pat. Nos. 3,850,752; 4,255,329; 4,233,402; and 4,208,479.

For performing the assay, kits can be provided where the various reagents are combined in predetermined amounts in combination with various ancillary materials for combination with the sample or for other uses in the assay. In view of the wide spectrum of protocols and reagents, a wide variety of kits may be prepared. In general, where the method involves the addition of particles, the kits will include particles which have a ligand bound to the particle, either covalently or non-covalently. Also, there may be a label bound to the surface of the particle or dispersed therein, particularly a dye, which may be colored in the visible range. The following references describe various labeling methods: chemiluminescence (Maier, et al., U.S. Patent No. 4,104,029); colored particles (Campbell, et al., U.S. Pat. No. 4,703,017); colorable particles (deJaeger, et al., U.S. Pat. No. 4,837,168); or fluorescence (Langone and Van Vunakis, eds, Methods in Enzymology 74; Part C (1981)). Radioisotopes may also be used to label the particles. (See, e.g., Langone and Van Vunakis, eds, Methods in Enzymology 70; Part A (1980), which describes radioisotopic labeling methods.) In other instances, the particle may also be labelled with an enzyme.

Where particles are not to be included, the reagents will normally involve labelled receptors or ligands, where the labels provide for a detectable signal and may provide for the inhibition of migration of the particles present in the assay medium. In addition to the labelled reagents, there may be ancillary reagents such as buffers, stabilizers, detergents, and as appropriate substrates for enzymes, bulking agents, and the like. Also likely to be included in such kits would be the wicking material, prepared in strip form or some other convenient shape. In preferred embodiments of the invention, the assay kit contains desiccant material; more preferably, the desiccant material is contained within the assay device or housing.

The invention can be better understood by way of the following examples which are representative of the preferred embodiments thereof, but which are not to be construed as limiting the scope of the invention.

EXAMPLE I

Lyme Antibody Assay

Extracted Lyme antigens are prepared from *Borrelia burgdorferi* strain B-31 purchased from the American Type Culture Collection. The microorganism was grown in modified BSK II Medium as described below. It was incubated at 33°C and harvested by centrifugation. The harvested cells were washed three times with phosphate buffered saline (pH 7.4) containing 5 mM $MgCl_2$, then extracted with 2% sarcosyl in 10 mM tris buffered solution (pH 8.2) containing 1 mM EDTA. This extraction procedure was carried out until all the sarcosyl soluble material was extracted. The insoluble material was further blended with an Omni mixer. The blended material is hereinafter referred to as the "Lyme antigens". It should further be noted that the same Lyme antigens or Lyme antigens from the same source may be used to detect Lyme-specific antibodies in a variety of mammals, including cattle, dogs, cats and humans.

EXAMPLE II

Preparation of Conjugates

While latex particles are used in the presently-described experiments, it should be appreciated that other particles may be used successfully. In addition, while microparticles that are not conjugated with ligands are added to ligand-particle conjugates in various examples, it should be understood that there are various embodiments of the present invention in which such admixing is not necessary to the practice the present invention.

Uniform latex particles ("ULPs") are, in general, extremely uniform spheres of small diameter. Typical diameters range from less than about 0.1μm to about 100 μm. Particles smaller than 5 μm are usually prepared by emulsion polymerization.

a. Preparation of Ligand-Particle Conjugates

The basic process of ligand-particle conjugation, for example, via simple adsorption or covalent binding, is well known in the art, as is the use of colored latex particles, which increase the resolution and readability of assays. Various procedures are described, in general terms, in Bangs, L.B., "Uniform Latex Particles," presented at a workshop at the 41st National Meeting, Amer. Assoc. Clin. Chem., 1989, and available in printed form from Seragen Diagnostics Inc., Indianapolis, IN; or Galloway, R.J., "Development of microparticle tests and immunoassays," Seradyn, Inc., Indianapolis, IN. These articles, and references cited to therein, are hereby incorporated by reference.

One method of preparing coated latex particles, for example, is the adsorption method. In general terms, one should: 1) utilize pure reagents; 2) clean the particles prior to coating; and 3) determine the quantitative surface coverage of the particle and the ligand chemistry.

For example, ligand-latex conjugates ("L-latex") may be prepared according to the following method: in the simplest case, the appropriate ligand is dissolved in a buffer

solution, added to a latex suspension, and stirred for times ranging from a few minutes to more than 24 hours. After equilibration, the latex is centrifuged and the supernatant containing any unadsorbed ligand is discarded. The latex is re-suspended in fresh buffer and centrifuged; the supernatant is again discarded. These steps are to be repeated until the latex is determined to be washed free of any residual unadsorbed ligand. At this juncture, the latex coating process may be complete and the latex ready to use in latex agglutination assays.

Covalent coupling involves the permanent or covalent binding of a ligand or other material to the latex particle surface. If covalent coupling is the method of choice, one must first couple the ligand to the latex particles, then maintain the stability of the latex particle suspension, followed by preventing the protein from becoming denatured. (For a general discussion of covalent coupling techniques, and citation to more detailed references, see Bangs, L.B., "Uniform Latex Particles," which has been incorporated herein by reference.)

While the foregoing discussion is in the context of latex particles, it will be appreciated that other particles, including, without limitation, naturally-occurring (e.g. plasmids) or synthetic particles (e.g. polymers), and metal colloids or particles (e.g., gold sol particles), may be used. These particles and their methods of extraction or preparation are well known in the art.

b. Preparation of BSA-Latex Conjugates

Preparation of bovine serum albumin - latex conjugates ("BSA-latex") is similar to ligand-latex (L-latex) preparation, as described above, except that no ligand is used in the preparation, and BSA is used instead. Alternatively, other proteins may be used in place of the BSA, such as other albumins (including lactalbumin), casein, globulin, non-specific immunoglobulin (which does not participate in the antigen-antibody reaction), and the like that can prevent nonspecific binding.

c. Mixture of Ligand-latex and BSA-latex

L-latex and BSA-latex are mixed together in varying ratios, depending upon the test to be performed. For example, in preparing mixtures for use as set out in some of the Examples, L-latex and BSA-latex were mixed in approximately a 2:1 ratio to a 5:1 ratio, volume to volume, for use in the assays. Depending upon the nature of the assay, the ratios can vary substantially, with greater amounts of protein-labeled latex resulting in greater reduction of nonspecific binding. The amount of latex (or other particle) that does not have ligand attached can be any amount that is effective to appreciably decrease nonspecific binding, or false positives. Such amounts are readily determined by obvious empirical methods.

d. Mixture of Ligand-latex and "Naked" latex

L-latex and naked latex are mixed together in varying ratios, depending upon the test to be performed, as noted above in regard to Ligand-latex/BSA-latex mixtures. As noted above, the ratios of L-latex to naked latex can vary substantially, with greater amounts of protein-labeled latex resulting in greater reduction of nonspecific binding. The amount of latex (or other particle) that does not have ligand attached can be any amount that is effective to appreciably decrease nonspecific binding, or false positives. Such amounts are readily determined by obvious empirical methods.

EXAMPLE III

Preparation of *Borrelia* Conjugate

10 ml of blue carboxylated latex (preferably about 0.4μ in diameter) having 50 mg/ml solid, obtained from Magsphere, is diluted to 0.5 mg/ml in 10% sucrose and 20mM MES buffer, pH 5.5. Add Sulfo-NHS (Pierce) and EDAC (Sigma, St. Louis, MO) to a final concentration of 1.087 mg/ml and 2 mg/ml, respectively. Mix by end-to-end rotation for about 30 minutes. The activated latex is washed five times with 10% sucrose, 0.02% SDS in 20mM MES, pH 6.5, in an amicon

concentrator with 0.2μm nylon membrane. Re-suspend to approximately 50 mg/ml with 10% sucrose, 2mM MES (pH 6.5), and sonicate four times for 15 seconds each time.

About 0.5 mg of extracted *Borrelia burgdorferi* antigens are mixed in 0.01% SDS, 2mM MES buffer (pH 6.5), containing 10% sucrose and the 500 mg of activated latex suspension. The latex and extracted *Borrelia burgdorferi* are allowed to react for two hours at 22°C.

To neutralize surface carboxyl groups not bound to the extracted *Borrelia burgdorferi* antigens, a solution of Tris buffer (pH 8.5), is added to 20mM, followed by a solution of casein (Sigma) and Zwittergent 3-12 (Calbiochem, San Diego, CA) of 0.5% and 0.1%, respectively. The *Borrelia burgdorferi*-latex is diluted to a desired concentration containing 20% sucrose, 20mM Tris (pH 8.5), 0.1% Zwittergent 3-12, 0.5% casein, and 0.3% BSA-latex, and is ready for printing onto the solid support or matrix.

EXAMPLE IV

Reagent Preparation

1. Preparation of BSK-II Medium

To 1 liter of fortified RPMI-1640 without glutamine add the following and mix well: 5 g Neopeptone (Difco) and 50 g BSA (Sigma A-4503; St. Louis, MO). Dissolve the neopeptone and BSA completely and then add the following: 2g TC Yeastolate (Difco); 6g HEPES (Sigma); 0.7g sodium citrate; 5g glucose; 0.8g sodium pyruvate; 0.4g N-acetylglucosamine (Sigma); and 2.2g sodium bicarbonate. Adjust the pH to 7.6-7.8.

Modified BSK-II medium was prepared according to the method set forth by Barbour, A.G., in Yale J. B. Med. 57: 521-525 (1984), which is incorporated herein by reference. The modified medium was prepared from fortified RPMI-1640 without glutamine and 7% gelatin.

2. Inoculation and Harvesting of *Borrelia* Cultures

The equipment used included a 2.2 L Erlenmeyer flask; a vacuum equipped with a trap; 0.2 micron 1 liter filter units (Nalgene 90mm diameter); and a laminar flow hood. The medium was inoculated with *Borrelia* according to appropriate, safe laboratory procedures. The media flasks are incubated at 33-35°C overnight as a precaution, to check for contamination. Signs of contamination include the appearance of turbidity and/or an acidic pH shift.

EXAMPLE V

Assay Preparation and Procedures

1. Preparation of solid phase

One example of the use of the present invention in a solid phase assay format may proceed essentially as follows. The illustrated reaction device consists of a solid -- typically plastic -- housing containing a solid support for the assay. Generally, a membrane strip such as nitrocellulose is used; preferably, an immunochromatographic strip is used. For example, the right end of the membrane provides contact with the sample well. The sample well contains an absorbent pad which provides an even flow of the sample fluid from right to left along the membrane. Mobile particles are applied to a first zone of the membrane -- for example, latex beads -- which particles are conjugated with the appropriate ligand or antiligand. For example, antigens extracted from *Borrelia burgdorferi* were used in one experiment described herein. Various methods of isolating and preparing viral or bacterial antigens are known in the art. For example, and without limitation, Hepatitis A antigen may be prepared according to the method of Markus, et al., as described in U.S. Pat. No. 4,301,249, which is incorporated herein by reference.

In a second zone on the membrane, ligand or antiligand is immobilized. A third reagent may be used as well; for example, such an agent may be immobilized in a zone on the solid support. This agent may be capable of binding particles that migrate from the first zone after sample is added. This

third agent can act as a procedural control and may serve to indicate that the assay is complete, or that it has been properly performed, if, for example, a detectable response occurs in the zone in which the third agent is immobilized.

In a "typical" chromatographic assay test procedure, a liquid specimen is applied to the solid support proximal to the first zone. As the fluid moves via capillary action to the first zone of the membrane, it mobilizes the mobile particles. The fluid continues to move the particles across the membrane to the next zone or zones. If analyte is present in the sample, a "sandwich" of solid phase-antigen/analyte/antigen-conjugated particles is formed and a detectable result occurs. As the fluid continues to move the particles across the membrane, the fluid/particles come into contact with the third reagent, which is immobilized on the support. A detectable response should then occur in that zone, indicating that the test is valid, or that the test is complete.

2. Test Procedure

Place the solid phase assay (or membrane alone) on a well-lighted, level surface. Place one drop (about 50μL) of liquid sample in/on the sample application area proximal to the first zone. The application area may also contain a buffer solution to further promote assay performance. One example of such a buffer comprises 2% casein and 0.5% Zwittergent in 0.1M Tris (pH 8.0). Alternatively, the sample may be mixed with another substance -- e.g., saline solution -- prior to administration of the sample or sample mixture to the sample application area. Immediately thereafter, add several (preferably about 6) drops of developer solution to the sample application area. A useful developer solution contains the following, for example, in aqueous solution: 1% Zwittergent, 1% Triton x-100, 0.02% SDS, 0.2% NaN_3, and 50mM Tris buffer (pH 8.5).

Allow the test to run for about 10 minutes and then read the results. Although a signal may appear in the third zone

before the 10 minutes elapse, the results are likely to be read with greater accuracy if one waits the full 10 minutes. After the appropriate amount of time elapses, provided the appropriate signal is detected in the approprate zone or zones, then the assay results may be read. For example, if a symbol appears in the third zone, then the test results may be interpreted as valid and negative. If a symbol appears in the second and third zones, then the results may be interpreted as valid and positive.

Control solutions may further be provided for the purpose of comparison with assay results or to test reagent viability. For example, a positive control solution may comprise about 0.5mg/ml purified rabbit anti-Lyme antibody in 2mg/ml of rabbit gamma globulin and 50mM Tris (pH 8.0). A negative control may comprise 2mg/ml of rabbit gamma globulin in 50mM Tris (pH 8.0). These solutions may be provided in a kit form with any or all of the reagents and assay components disclosed herein.

Although the invention has been described in the context of particular embodiments, it is intended that the scope of coverage of the patent not be limited to those particular embodiments, but be determined by reference to the following claims.

WE CLAIM:

1. An analytical device for the detection or determination of an analyte antibody in a bodily fluid comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, said layer including:

a) a sample application zone;

b) a conjugate zone containing antigen bound to mobile particles; and

c) a detection zone containing immobilized antigen wherein the antigen is the same in both the conjugate and detection zones and is an antigen that binds with the analyte antibody, the liquid sample is capable of moving from the sample application zone through the conjugate zone and on to the detection zone, and if said analyte antibody is present in the sample it is detected in the detection zone.

2. A device of claim 1, wherein the mobile particles are colored plastic particles or a metal sol.

3. A device of claim 2, wherein the antigen will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism.

4. A device of claim 3, wherein the mobile particles are colored polystyrene microparticles.

5. A device of claim 4, wherein the layer is made from nitrocellulose.

6. A method employing the device of claim 1, which comprises adding sample suspected of containing the analyte antibody to the sample application zone and waiting for sufficient time for the sample to traverse the layer through the detection zone, and reading the results in the detection zone.

7. A method of claim 6, wherein the mobile particles are colored plastic particles or a metal sol.

8. A method of claim 7, wherein the antigen used will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism.

9. A method of claim 8, wherein the mobile particles are colored polystyrene microparticles.

10. A method of claim 9, wherein the layer is made from nitrocellulose.

11. A process for the determination of the presence or concentration of an analyte antibody in a fluid which comprises:

a) contacting a sample of the fluid with a first antigen for the analyte antibody, wherein the first antigen is labelled, in order to form a soluble complex between the first antigen and the analyte antibody;

b) contacting the soluble complex with a second antigen, wherein said second antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex of the first antigen, the analyte antibody, and the second antigen;

c) separating the solid phase from the fluid sample and the unreacted, first antigen;

d) measuring either the first, labelled antigen associated with the solid phase of the unreacted amount of the first, labelled antigen;

e) relating the amount of first, labelled antigen measured for a control sample prepared in accordance with steps a) through d), said control sample being free of the analyte antibody, to determine the presence of the analyte antibody in said fluid sample, or relating the amount of first, labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with steps a) through d) in order to determine the concentration of the analyte antibody in the fluid sample;

wherein both the first and second antigens are the same before they are labelled or attached to the solid phase; respectively.

12. A process of claim 11, wherein the label is an enzyme or a radioisotope.

13. A process of claim 12, wherein the antigen reacts with antibodies that in turn react with epitopes of *Borrelia burgdorferi*.

14. A process of claim 13, wherein the label is alkaline phosphatase and the solid phase is a bead, the inner walls of a test tube or the wells of a microtitre plate.

15. A process of claim 14, wherein the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

16. An assay kit comprising a first antigen bound to a solid phase insoluble in the fluid to be tested and a reagent containing a second antigen bound to a label, wherein the solid phase and the reagent are present in sufficient amounts to perform at least one assay for analyte antibody in the fluid, and wherein the first and second antigens are the same before they are bound to the solid phase or are labelled, respectively.

17. An assay kit of claim 16, wherein the label is an enzyme or a radioisotope.

18. An assay kit of claim 17, wherein the antigen is an antigen that binds to antibodies which in turn bind to epitopes of *Borrelia burgdorferi*.

19. An assay kit of claim 18, wherein the label is an enzyme.

20. An assay kit of claim 19, wherein the solid phase is a non-chromatographic device.

21. An assay kit of claim 20, wherein the label is alkaline phosphatase and the solid phase is a plastic bead, the inner walls of a test tube or the wells of a microtitre plate.

22. A process for the determination of the presence or concentration of an analyte antibody in a fluid comprising the steps:

a) simultaneously contacting a sample of the fluid with a first and second antigens, wherein the first antigen is bound to a solid phase insoluble in the fluid and the second antigen is labelled and provided in a measured in order to form a insoluble complex between the first and second antigens and the analyte antibody;

b) separating the solid carrier from the fluid sample containing unreacted second, labelled antigen;

c) measuring the amount of the second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen;

d) relating the amount of second labelled antigen with the amount of labelled antigen measured for a control sample prepared in accordance with steps (a) through (c), said control sample known to be free of analyte antibody, to determine the presence of analyte antibody in the fluid sample, or relating the amount of labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amount of analyte antibody prepared in accordance with steps a) through c) to determine the concentration of the analyte antibody in the fluid sample;

wherein the first and second antigens are the same before they are bound to a solid phase and labelled, respectively.

23. A process of claim 22, wherein the label is an enzyme or radioisotope.

24. A process of claim 23, wherein the first and second antigen bind to antibodies that in turn bind to epitopes of *Borrelia burgdorferi*.

25. A process of claim 24, wherein the label is alkaline phosphatase and the solid phase is a plastic bead, the inner walls of a test tube, or the wells of a microtitre plate.

26. A process of claim 24, wherein the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

27. A process for the determination of the presence or amount of an analyte antibody in a fluid sample comprising the steps:

a) contacting a sample of the fluid with a first antigen, wherein the first antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex between the first antigen and the analyte antibody;

b) separating the fluid sample containing the unreacted analyte antibody from the insoluble complex of the analyte antibody and first antigen;

c) reacting a measured amount of a second, labelled antigen with the insoluble complex of the first antigen and the analyte antibody to form an insoluble complex composed of the first and second antigens and the analyte antibody;

d) separating the solid phase from the unreacted second, labelled antigen;

e) measuring either the amount of second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen;

f) relating the amount of second, labelled antigen measured with the amount of labelled antigen measured for a control sample prepared in accordance with steps a) through e) being known to be free of the analyte antibody to determine the presence of analyte antibody, or relating the amount of labelled antigen measured in the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with steps a) through e) to determine the concentration of analyte antibody in the fluid sample;

wherein the first and second antigens are the same before they are bound to a solid phase or labelled, respectively.

28. A process of claim 27, wherein the label is an enzyme or a radioisotope.

29. A process of claim 28, wherein the first and second antigens are both antigens that complex with epitopes of the *Borrelia burgdorferi* microorganism.

30. A process of claim 29, wherein the label is alkaline phosphatase.

31. A process of claim 30, wherein the solid phase is a bead, the inner walls of a test tube, or the wells of a microtitre plate.

32. A process of claim 30, wherein the solid phase is a non-chromatographic device.